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TITLE: Exploiting Inhibitory Siglecs to Combat Food Allergies

PARTNERING INVESTIGATOR: Matthew Macauley, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute
La Jolla, CA 92037

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6. AUTHOR(S)
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Matthew Macauley, PhD – Partnering PI

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5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Scripps Research Institute
10550 N. Torrey Pines Road
La Jolla, CA 92037

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13. SUPPLEMENTARY NOTES
Please note that for the coming year, Dr. James Paulson (TSRI) will be taking over as the Partnering PI, as Dr. Macauley has moved to the University of Alberta (Edmonton, Alberta, Canada). Dr. Macauley will continue to participate in the project as a sub-contract from TSRI. An official request for this change in PI was submitted as a separate package.

14. ABSTRACT
During this first year of the award, we were able to produce important data, develop a new tool to track allergen-specific B cells, and generate two humanized mouse models. We demonstrated that Ah2 STALs targeting CD22 on B cells can prevent IgE production and allergic reactions with only a single injection in mice. We also demonstrated that Ah2 STALs targeting CD33 on human mast cells and basophils can prevent degranulation in cell culture experiments. A novel tool, Ah2 tetramer, was produced to quantify numbers of allergen-specific B cells in mice. A model to test Ah2 STALs as a therapy was developed in which naïve mice were adoptively transferred memory B and T cells from peanut allergic mice. The resulting mice make robust levels of Ah2-IgE and experience anaphylaxis upon challenge. This model will be used to test STALs as a therapy to deplete memory B cells. Furthermore, two novel transgenic mouse models were generated, one expresses human CD22 on B cells and the other expresses human CD33 on mast cells. These models are vital to our proposed research.

15. SUBJECT TERMS
Food allergy; peanut allergy; Siglec; IgE; Ara h 2; CD22; CD33; mast cell; basophil

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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1. **INTRODUCTION:**

In this pre-clinical, translational project, we will utilize mouse models, human B cells, and human mast cells and basophils to assess the ability of Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to induce immunological tolerance to peanut allergens. STALs are bioengineered nanoparticles that co-display a selected antigen and high affinity Siglec ligand. STALs targeting the Siglec CD22 on B cells induce antigen-specific B cell tolerance through deletion of the B cells recognizing the antigen. Applying this approach to animals with an existing peanut allergy will allow us to deplete memory B cells responsible for producing IgE, and establish a novel therapeutic strategy for food allergies. STALs targeting the human Siglec CD33 will be used to desensitize mast cells. This approach will be investigated as a therapeutic strategy for preventing acute allergic reactions, allowing for tolerizing doses of antigen to be delivered safely. By exploiting the inhibitory functions of CD22 on B cells, and CD33 on mast cells and basophils, our primary objectives are (1) to develop a novel prophylactic and therapeutic approach for peanut allergy and (2) to develop a targeted approach to prevent mast cell and basophil degranulation to peanut allergens.

2. **KEYWORDS:**

Food allergy; Peanut allergy; Siglec; CD22; CD33; STAL; nanoparticle; Ara h 2; mast cell; basophil; B cell

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

  - Specific Aim 1: Establish the therapeutic potential of Ara h 2 STALs targeting CD22 to abrogate peanut allergies.
    - Major Task 1: Determine optimal conditions to induce B cell tolerance to Ara h 2 and whole peanut extract in a prophylactic mouse model. *Target date*: Months 1-12; *percentage of completion*: 100% (completed)
    - Major Task 2: Use Ara h 2 STALs to induce tolerance by deletion of memory B cells. *Target date*: Months 10-30; *percentage of completion*: 15%
    - Major Task 3: Determine translatability of STALs to human CD22 and human B cells. *Target date*: Months 5-24; *percentage of completion*: 20%
  - Specific Aim 2: Demonstrate the applicability of Ara h 2 STALs targeting CD33 to prevent mast cell- and basophil-mediated allergic responses to peanut allergen.
    - Major Task 1: Determine inhibitory effects and longevity of inhibition using LAD-2 mast cells. *Target date*: Months 1-7; *percentage of completion*: 100% (completed)
    - Major Task 2: Determine inhibitory effects and longevity of inhibition using human basophils. *Target date*: Months 7-18 ; *percentage of completion*: 15%
    - Major Task 3: Determine preventive effects of STALs targeting CD33 on mast cells in vivo in allergic mice. *Target date*: Months 6-30; *percentage of completion*: 20%
    - Major Task 4: Determine therapeutic utility of STALs targeting CD33 and CD22 simultaneously in allergic mice. *Target date*: 18-36; *percentage of completion*: 0%

- **What was accomplished under these goals?**

Please note, this award is for a Partnering PI project with the PI, Michael Kulis at the University of North Carolina (UNC) and Matthew Macauley at The Scripps Research Institute (TSRI) and each have contributed to the progress of the project. In the accomplishments listed below, we have noted which PI (Kulis/UNC or Macauley/TSRI) was involved in specific experiments conducted during the reporting period.

Specific Aim 1: Establish the therapeutic potential of Ara h 2 STALs targeting CD22 to abrogate peanut allergies

Major Task 1 - Determine optimal conditions to induce B cell tolerance to Ara h 2 and whole peanut extract in a prophylactic mouse model.

Significant Results and Achievements: IACUC protocols were written and approved at UNC, and then submitted and received approval from USAMRMC ACURO. The major peanut allergen, Ara h 2, was proposed as the model antigen to demonstrate key concepts in preventing and treating peanut allergy. Purified Ara h 2 (Ah2) was produced and shipped from UNC to TSRI where Ah2 was conjugated to lipid and saved as a frozen stock. The frozen stock was validated at TSRI for making Ah2 STALs with CD22 ligand (CD22L), and will be used for subsequent experiments throughout the project period.

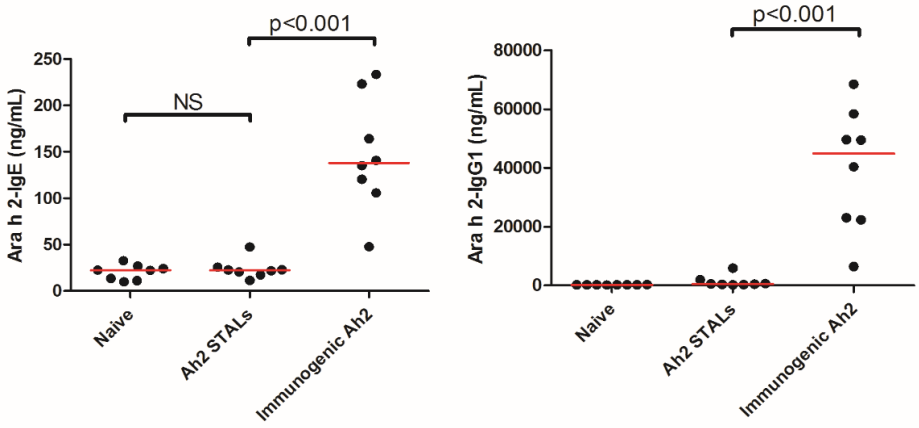

Figure 1. Ara h 2-specific IgE and IgG1 levels in serum. Mice were given PBS (Naïve), Ah2 STALs, or Immunogenic Ah2, then sensitized with peanut plus cholera toxin or sham sensitized with PBS (Naïve). Blood was collected one week after sensitization for serum IgE and IgG1 measurements. Significantly lower levels of Ara h 2-specific IgE and IgG1 were found in mice treated with Ah2 STALs compared to mice treated with immunogenic Ah2 liposomes. These findings indicate that Ah2 STALs deplete naïve B cells thus preventing the production of Ah2-specific IgE and anaphylaxis upon allergen challenge.

The initial experiment was designed to test the Ah2 STALs with mouse CD22L in a prophylactic approach in mice. The results generated at UNC demonstrated that one i.v. injection of Ah2 STALs prevented Ah2-IgE and Ah2-IgG production following the peanut plus cholera toxin sensitization (**Figure 1**), and ultimately prevented anaphylaxis with Ah2 challenge. This confirms that the STALs work appropriately and the model can be used to track Ah2-specific B cell responses in further experiments. Results from a similar series of experiments were published in the *Journal of Allergy and Clinical Immunology* (Impact Factor: 12.485) using preliminary data that was submitted with the DoD proposal.

We are very interested in tracking Ah2-specific B cells to understand the mechanism of preventing IgE production. To do this, we proposed working with a collaborator, Justin Taylor at the Fred Hutchinson Cancer Research Center, who is an expert in identifying very rare populations of B cells in mice. Dr. Taylor successfully produced an Ah2 tetramer by linking biotinylated Ah2 to streptavidin that is tagged with an APC fluorophore. We have since performed experiments at UNC to demonstrate that we can identify Ah2-specific B cells in mice, where peanut-sensitized mice have many more Ah2-specific B cells than naïve mice (**Figure 2**). This novel tool will be critical to further mechanistic studies in Aim 1.

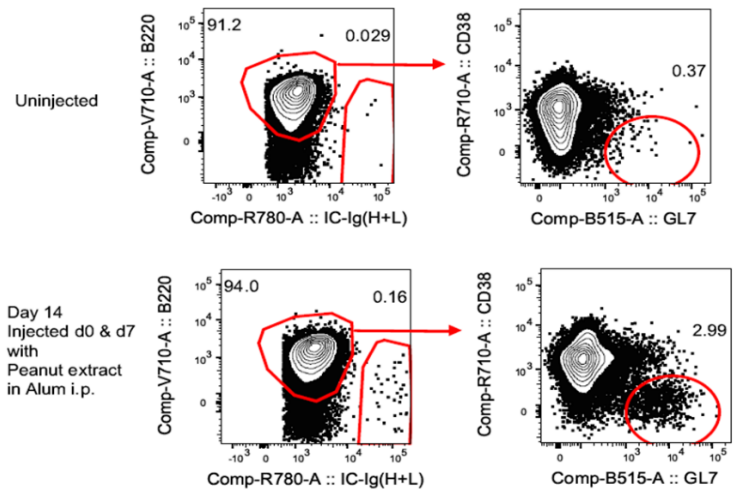

Figure 2. Identification of Ah2-specific B cells in naïve (uninjected) mice and peanut immunized mice. The top panels show naïve mouse B cells with very few germinal center (CD38- GL7+) Ah2-specific B cells (0.37% of B220+ cells). The bottom panels show immunized mice have ~3 times as many B220+CD38-GL7+ cells (2.99%) as the naïve mice.

Major Task 2: Use Ara h 2 STALs to induce tolerance by deletion of memory B cells.

Significant Results and Achievements: A major focus of Aim 1 is to move beyond prevention of peanut allergy and into the realm of therapy. We proposed to test this first in mice with conferred memory responses to Ah2. To do this, we needed to develop a mouse model in which memory T and B cells were adoptively transferred into naïve mice. At UNC, BALB/cJ mice were sensitized to peanut using the typical peanut plus cholera toxin protocol, then splenocytes (containing memory T and B cells) were harvested and transferred into naïve BALB/cJ mice. Mice were then given either PBS (sham treated) or Ah2 injections to test memory recall responses. Mice were bled for Ah2-IgE quantification and later challenged with Ah2 to assess anaphylaxis. The Ah2 injections boosted Ah2-IgE and led to anaphylaxis whereas this did not happen in mice given PBS only (**Figure 3**). This model has been repeated twice and will serve as our initial model to test the Ah2 STALs with mouse CD22L as a therapeutic to blunt memory B cell responses. The Ah2 Tetramer will also be used in this model to track memory B cell numbers and functional markers.

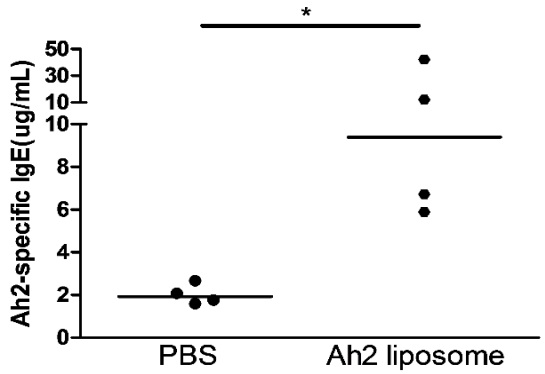

Figure 3. Ah2-specific IgE in mice with adoptively transferred memory B and T cells that were then immunized with Ah2. Mice given only PBS did not generate a large recall response as evidenced by low levels of Ah2-IgE, whereas mice given Ah2 liposomes had large increases in Ah2-IgE demonstrating that memory B cells were activated and able to produce Ah2-IgE.

Major Task 3: Determine translatability of STALs to human CD22 and human B cells

Significant Results and Achievements: IACUC protocols were written and approved at TSRI and received approval from USAMRMC ACURO for studies conducted at TSRI. A major question is the ability of STALs to be translated into humans. To begin addressing this question, Dr. Macauley produced transgenic mice expressing human CD22 on B cells. These mice were produced and human CD22 expression confirmed on the B-cells (**Figure 4A**) at levels only modestly lower than genuine human peripheral blood B-cells (**Figure 4B**). Several cohorts of WT and hCD22 transgenic mice were sensitized with crude peanut extract according to the protocol developed and implemented by Dr. Kulis. We find that hCD22 mice mount similar level of anti-Ara h 2 antibodies compared to WT mice (**Figure 4C**), and have a similar degree of anaphylactic response as WT mice following a challenge with peanut extract (**Figure 4D**). This work was recently published in the *Journal of Immunology* (Impact Factor: 4.92) as a part of work describing this mouse model, which concluded the hCD22 is sufficient to functionally substitute for mCD22 in most aspects of B-cell biology. This mouse model sets up future experiments using human CD22L on Ah2 STALs as a therapeutic approach in mice expressing the human CD22 receptor.

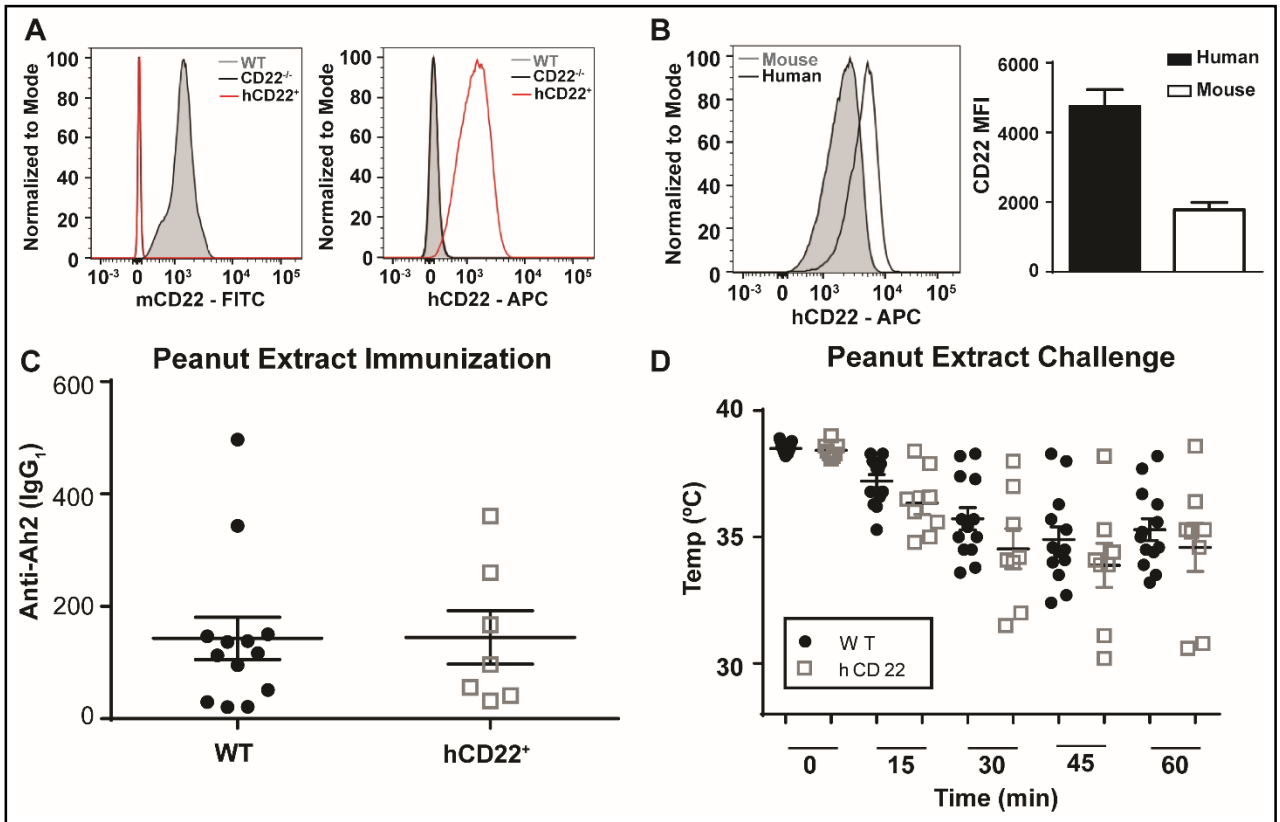

Figure 4: Development of a hCD22 transgenic mouse. (A) Expression of mCD22 (left) and hCD22 (right) on WT ($mCD22^{+}hCD22^{-}$; grey), hCD22 ($mCD22^{-/-}hCD22^{+}$; red), and CD22KO ($mCD22^{-/-}hCD22^{-}$). **(B)** Comparison of hCD22 expression on genuine primary human peripheral blood B-cells and murine hCD22 transgenic B-cells. On the right is a histogram showing the relative expression levels in multiple patients/mice. **(C,D)** WT or hCD22 transgenic mice were orally sensitized with crude peanut extract and cholera toxin for four consecutive weeks, then challenged with crude peanut extract a week later intraperitoneally. **(C)** Antibody responses to the major peanut allergen (Ara h 2) in mice following the four sensitizations. **(D)** Anaphylactic response in mice following the challenge. No differences in antibody responses or temperature decreases were observed between the two types of mice.

Specific Aim 2: Demonstrate the applicability of Ara h 2 STALs targeting CD33 to prevent mast cell- and basophil-mediated allergic responses to peanut allergen

Major Task 1: Determine inhibitory effects and longevity of inhibition using LAD-2 mast cells.

Significant Results and Achievements: The first major task was to optimize the ratio of Ah2 and human CD33L on STALs to generate fully hypoallergenic STALs. At TSRI, several formulations of STALs were prepared and ultimately tested on the human mast cell line, LAD-2. An ideal ratio was found that led to essentially no degranulation from the LAD-2 cells, whereas Ah2 liposomes without the human CD33L led to robust degranulation (**Figure 5A**).

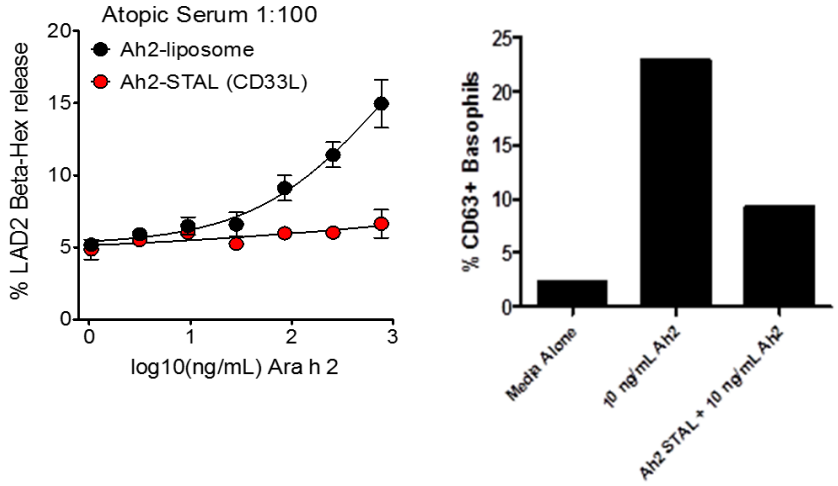

Figure 5. Ah2 STALs targeting CD33 on human mast cells and basophils. Left panel (A) shows Ah2-STALs (red circles) does not cause beta-hex release (i.e. degranulation), whereas Ah2-lioposomes (black circles) cause large amounts of degranulation. Right panel (B) demonstrates that 10 ng/mL Ah2 causes CD63+ degranulation of human basophils whereas when basophils are first treated with the Ah2 STAL then 10 ng/mL Ah2 there is a >50% reduction in degranulation, which is still somewhat higher than the media alone control.

Major Task 2: Determine inhibitory effects and longevity of inhibition using human basophils.

Significant Results and Achievements: IRB approval was obtained at UNC and then with USAMRMC HRPO. At UNC, an initial pilot study was conducted using the whole blood basophil activation assay. The data look promising as STAL treated basophils degranulate less than untreated cells when stimulated with Ah2 (**Figure 5B**). Further experiments, including additional controls, are planned for the human basophil assay to better optimize the STALs treatments.

Major Task 3: Determine preventive effects of STALs targeting CD33 on mast cells in vivo in allergic mice.

Significant Results and Achievements: Appropriate IACUC approval was obtained at TSRI and then with USAMRMC ACURO. In order to test the efficacy of Ah2 STALs targeting CD33 *in vivo*, transgenic mice expressing the human CD33 receptor on mast cells needed to be generated. At TSRI, transgenic mice were produced and expression of human CD33 on mast cells was verified (**Figure 6**). These transgenic mice will be a critical tool moving forward with Ah2 STALs, with the aim of silencing mast cells in response to peanut challenge.

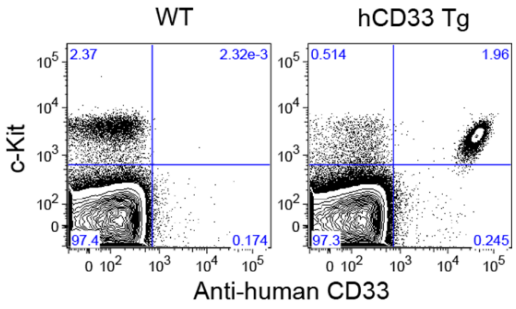

Figure 6. Mast cells in transgenic mice express human CD33. Left panel shows c-Kit+ mast cells do not express human CD33 in wild-type (WT) mice, as expected. The right panel shows c-Kit+ mast cells do express human CD33 in the transgenic mice.

- **What opportunities for training and professional development has the project provided?**
  - At UNC, Dr. Kulis worked with Kelly Orgel (an MD/PhD student) to continue mentoring her on basic science experiments and techniques. Additionally, results from the CD22 STALs in mice were presented by Kelly Orgel during the UNC Pediatric Day of Scholarship, in which she was awarded with the "Best basic science abstract".
  - At TSRI, through one-on-one mentoring between Dr. Macualey and Shiteng Duan, Shiteng has become very efficient in all aspects needed for the proposed project including mouse handling, liposome preparation, and in vitro assays, such as ELISAs. Shiteng and Kevin Worrell participated in groups meetings, regularly presenting their research updates. Dr. Macauley worked closely with Joana Juan to carry out the mouse genotyping and coordinate the mouse experiments. Shiteng also orally presented his research at two departmental meetings at TSRI: once in the Department of Immunology and Microbial Sciences, and once in the Department of Molecular Medicine.

- **How were the results disseminated to communities of interest?**
  - Nothing to Report.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - At UNC, we will continue to work with the Ara h 2 tetramer to examine B cell frequencies in mice treated with Ara h 2 STALs. We will also determine the efficacy of Ah2 STALs targeting memory B cells in the adoptive transfer model developed during the reporting period. Furthermore, we will assess the capacity of Ah2 STALs in depletion of human B cells from peanut allergic subjects. Finally, we will continue performing studies using human basophil assays to assess CD33 STALs in preventing degranulation.
  - At TSRI, the hCD33 mice will be used in functional studies to determine if hCD33L STALs can prevent an anaphylactic response (Major Task 3 in Aim 2). Shiteng Duan will continue collaborating with Dr. Kulis on the basophil assay.
  - At UAlberta, the hCD22 mice will be used in functional studies to determine if hCD22L STALs can prevent an anaphylactic response (Major Task 3 in Aim 1).

Dr. Macauley will continue provide Dr. Kulis with mCD22L STALs for studies in mice at UNC.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Two novel mouse models were developed by Dr. Macauley's group at Scripps. These are the humanized CD22 B cell mice and the humanized CD33 mast cell mice. These models provide a vital tool for scientists exploring the in vivo roles of CD22 and CD33, both in a basic scientific sense as well as applied.

- **What was the impact on other disciplines?**
  - Nothing to Report.

- **What was the impact on technology transfer?**
  - Nothing to Report.

- **What was the impact on society beyond science and technology?**
  - Nothing to Report.
  -

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  - There were no significant changes to the approach during the reporting period.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - Nothing to Report.

- **Changes that had a significant impact on expenditures**
  - At UNC, a postdoc was hired, which did not happen until approximately 8 months into the funding period. This likely decreased personnel costs for the reporting period at UNC.
  - At TSRI, since Dr. Macauley was moving institutions he had to hold off on hiring a postdoc for the project. However, by Dr. Macauley pulling some of the experimental weight, the objectives could be achieved.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to Report.
- **Significant changes in use or care of human subjects.**
  - Nothing to Report.
- **Significant changes in use or care of vertebrate animals.**
  - Nothing to Report.
- **Significant changes in use of biohazards and/or select agents.**
  - Nothing to Report.

6. **PRODUCTS:**
- **Publications, conference papers, and presentations**
  - **Journal publications.**

Human CD22 Inhibits Murine B Cell Receptor Activation in a Human CD22 Transgenic Mouse Model.
Bednar KJ, Shanina E, Ballet R, Connors EP, Duan S, Juan J, Arlian BM, **Kulis MD**, Butcher EC, Fung-Leung WP, Rao TS, Paulson JC, **Macauley MS**.

J Immunol. 2017 Sep 29. [Epub ahead of print]
PMID: 28972089
Yes, DoD Federal Funding was acknowledged.

Exploiting CD22 on antigen-specific B cells to prevent **allergy** to the major peanut allergen Ara h 2.
Orgel KA, Duan S, Wright BL, Maleki SJ, Wolf JC, Vickery BP, Burks AW, Paulson JC, **Kulis MD, Macauley MS.**
J Allergy Clin Immunol. 2017 Jan;139(1):366-369.e2 Epub 2016 Aug 20.
PMID:27554819
This paper used data submitted as Preliminary Data for the DoD proposal and went to press prior to receiving DoD Federal Funding, so funding from DoD was not listed.

- **Books or other non-periodical, one-time publications.**

Nothing to Report.

- **Other publications, conference papers, and presentations.**

Completed:

1. UNC Pediatric Scholarship Day
   April 20, 2017, UNC, Chapel Hill, NC
   Title: Exploiting CD22 on Ara h 2-Specific B-Cells to Prevent Allergy to the Major Peanut Allergen Ara h 2
   Authors: **Kelly Orgel**, Shiteng Duan, Brian Vickery, A. Wesley Burks, James Paulson, Matt Macauley, Michael Kulis

Accepted Abstracts (will be presented in the coming year):

1. American Academy of Allergy, Asthma, and Immunology Annual Conference
   March 2-5, 2018, Orlando, FL
   Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to reduce memory B cell responses to the major peanut allergen Ara h 2
   Authors: **L. Hardy**, K. Orgel, S. Duan, Soheila Maleki, A.W. Burks, J. Paulson, M. Macauley, M.Kulis

2. Gordon Conference on Food Allergy
   Jan 7-12, 2018, Ventura, CA
   Title: Targeting inhibitory Siglecs to prevent IgE dependent anaphylaxis
   Authors: **Shiteng Duan**, Corwin M. Nycholat, Matthew S. Macauley, Zhou Zhu, Bruce S Bochner, and James C. Paulson

3. Society for Glycobiology Annual Meeting
   November 5-8, 2017, Portland, OR
   Title: Development of a New Human CD22 Transgenic Mouse

Authors: **Matthew S Macauley**, Kyle J Bednar, Elena Shanina, Romain Ballet, Edward P Connors, Shiteng Duan, Joana Juan, Britni M. Arlian, Mike D Kulis, Eugene C Butcher, Wai-Ping Fung-Leung, Tadimeti S Rao, James C Paulson

4. American Chemical Society Spring National Meeting
   March 18-22, 2018, New Orleans, LA
   Title: Exploiting the Inhibitory Function of CD22 on B-cells to Prevent Antibody Responses
   Authors: **Matthew S. Macauley**, Britni M. Arlian, Kyle J. Bednar, Shiteng Duan, Wai-Ping Fung-Leung, Mike D. Kulis, Lakeya Hardy, Corwin M. Nycholat, Kelly A. Orgel, Lijuan Pang, James C., Paulson, Tadimeti S Rao

5. American Chemical Society Spring National Meeting
   March 18-22, 2018, New Orleans, LA
   Title: Siglecs: Putting the brakes on the immune system
   Authors: **James C. Paulson**, Britni Arlian, Shiteng Duan, Landon Edgar, Chika Kikuchi, Matthew Macauley, Corwin M. Nycholat, Lijuan Pang, Amrita Srivastiva

- **Website(s) or other Internet site(s)**
  - Nothing to Report.
- **Technologies or techniques**
  - Nothing to Report.
- **Inventions, patent applications, and/or licenses**
  - Nothing to Report.
- **Other Products**
  At TSRI, Dr. Macauley developed two transgenic mouse models – mice expressing either human CD22 on their B-cells or human CD33 on their mast cells

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	Michael Kulis, PhD
Project Role:	Initiating PI (UNC)
Researcher Identifier (e.g. ORCID ID):	0001-8092-7444
Nearest person month worked:	4
Contribution to Project:	As PI on the project, Dr. Kulis led and oversaw all of the work conducted at UNC. Specifically, Dr. Kulis was involved in experimental design, collaborating with Dr. Macauley at Scripps, data collection, data analysis, and troubleshooting.
Funding Support:	N/A

Name:	Rishu Guo, PhD
Project Role:	Research Scientist (UNC)

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Guo performed flow cytometry and cell culture experiments. These include work on the mouse B cells and human basophil assays.
Funding Support:	N/A

Name:	Lakeya Hardy
Project Role:	Graduate Student (UNC)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3
Contribution to Project:	Lakeya developed the adoptive transfer model. Specifically, she sensitized mice to peanut, harvested splenocytes, transferred the cells into naïve animals and then immunized with Ara h 2. She also contributed by running some ELISA experiments.
Funding Support:	N/A

Name:	Kelly Orgel
Project Role:	Graduate Student (UNC)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Kelly worked primarily on the use of Ah2 STALs targeting B cells to induce tolerance. Kelly performed many hands-on mouse procedures and peanut challenges. She also assisted with cellular studies, flow cytometry, and ELISA. Kelly performed the human CD33 basophil assays as well.
Funding Support:	N/A

Name:	Johanna Smeekens, PhD
Project Role:	PostDoc Scientist (UNC)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Smeekens assisted with hands-on mouse experiments, particularly the allergic challenge experiments. She helped to harvest splenocytes, stained cells for flow cytometry, and assisted with the human CD33 basophil assay experiments.

Funding Support:	N/A

Name:	Jada Suber
Project Role:	Graduate Student (UNC)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Jada helped with flow cytometry panel design for the Ah2 B cell tetramer experiments. Jada collected cells and stained them with these panels for later collection on the flow cytometer.
Funding Support:	N/A

Name:	Xiaohong Yue, MS
Project Role:	Research Associate (UNC)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3
Contribution to Project:	Xiaohong helped with routine technical work, including ELISA, cell preparation and counting, flow cytometry after cell culture, and preparation of reagents needed throughout the experiments.
Funding Support:	N/A

Name:	Matthew Macauley, PhD
Project Role:	Partnering PI (TSRI)
Researcher Identifier (e.g. ORCID ID):	0003-4579-1048
Nearest person month worked:	7
Contribution to Project:	Dr. Macauley provided guidance of project at TSRI as well as some experimental procedures. Dr. Macauley was responsible for getting animal protocols approved. Experimentally, Dr. Macauley conjugated Ah2 to lipid , for making of the STALs., and sensitized mice with the peanut allergen.
Funding Support:	N/A

Name:	Joana Juan
Project Role:	Research Assistant (TSRI)
Researcher Identifier (e.g. ORCID ID):	2-1463-3541
Nearest person month worked:	8

Contribution to Project:	Joana performed all the mouse genotyping and helped setup the appropriate mouse breeders. Joana also performed retro-orbital bleeds and analyzed antibody titers by ELISA
Funding Support:	N/A

Name:	Shiteng Duan
Project Role:	Grad Student (TSRI)
Researcher Identifier (e.g. ORCID ID):	1-7390-309X
Nearest person month worked:	7
Contribution to Project:	Shiteng developed the hCD33 trangenic mice. Shiteng also preparing the Ah2 STALs for use at TSRI and UNC, and helped challenge the mice with peanut extract and monitored the anaphylactic response.
Funding Support:	N/A

Name:	Kevin Worrell
Project Role:	Research Assistant (TSRI)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Kevin was responsible for synthesizing the Siglec ligands and conjugating these glycans to lipids for incorporation into STALs.
Funding Support:	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - For Dr. Kulis at UNC, nothing significant to report.
  - For Dr. Macauley, NIH grant R01AI118842 and R21AI128598 have been funded. Dr. Macauley will put 3 calendar months toward R21AI128598 and 4 calendar months toward R01AI118842 in the coming year. Given the situation with Dr. Macauley moving to University of Alberta and sub-contracting a portion of the work with Dr. James Paulson taking over as the partnering PI at TSRI, Dr. Macauley's effort on this project will decrease to approximately calendar months in the coming year. These changes have been submitted as a part of package sent for changing the partnering PI from Dr. Macauley to Dr. Paulson.

- **What other organizations were involved as partners?**

Justin Taylor, PhD
- **Organization Name:** Fred Hutchinson Cancer Research Center
- **Location of Organization:** Seattle, WA
- **Partner's contribution to the project:** Produced the Ara h 2 tetramers with purified Ara h 2 shipped from UNC and TSRI.
- **Financial support;** N/A

- **In-kind support** N/A
- **Facilities** N/A
- **Collaboration** Dr. Kulis, Dr. Macauley, and Lakeya Hardy worked with Dr. Taylor and his staff to develop and optimize tetramers
- **Personnel exchanges** project staff used materials provided by Dr. Taylor
- **Other.** N/A

Soheila Maleki, PhD

- **Organization Name:** United States Department of Agriculture
- **Location of Organization:** New Orleans, LA
- **Partner's contribution to the project:** Purified and provided the peanut allergen, Ara h 2.
- **Financial support;** N/A
- **In-kind support** N/A
- **Facilities** N/A
- **Collaboration** Dr. Kulis, worked with Dr. Maleki and her staff to provide the Ara h 2
- **Personnel exchanges** project staff (both UNC and TSRI) used materials provided by Dr. Maleki
- **Other.** N/A

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**

   A duplicative report will be submitted as tasks have been clearly marked with the responsible PI and research site.

   - **QUAD CHARTS:**

   N/A

9. **APPENDICES:**

   N/A